Genoepidemiology and Pathogenicity of Hepatitis G Virus in Japan

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KOBAYASHI, T., ISHII, M., NIITSUMA, H., KIKUCHI, K., SUZUKI, C., GAMA, H., KOBAYASHI, K., UENO, Y. and TOYOTA, T. *Genoepidemiology and Pathogenicity of Hepatitis G Virus in Japan*. Tohoku J. Exp. Med., 1997, **183** (2), 101-112 — A recently discovered non-A non-B hepatitis virus has been designated hepatitis G virus (HGV). Blood contamination has been proposed as its mode of transmission. We studied the genoprevalence of HGV in Japanese people at high risk. HGV was identified in serum by a reverse-transcription polymerase chain reaction. HGV was detected in 16.0% of intravenous drug users (IDUs) ($n=25$), 16.2% of those with tattoos ($n=37$), 10.9% of IDUs with tattoos ($n=55$), 5.7% of chronic hepatitis (CH)-C patients ($n=87$), and in none of the CH-B ($n=50$) or CH non-B non-C ($n=46$) patients. Serum alanine aminotransferase (ALT) levels of those infected with HGV alone ($n=3$) were all within normal range. In the patients with CH-C, serum ALT levels of those coinfecting with HGV were similar to serum ALT levels of those without HGV infection. A phylogenetic tree of isolated HGV clones showed that the HGVs of these subjects bore only a distant-resemblance to clones reported from Africa and North America, and that variation in the phylogenetic index of HGV clones was small. These results suggest that HGV clones from different areas have genetic heterogeneity and that HGV causes no or mild hepatitis. ——— hepatitis G virus; polymerase chain reaction; genotype; hepatitis

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The agent that induced hepatitis and that was passaged serially in primates originated from the serum of a 34-year-old surgeon, from whom it was obtained during the third day of jaundice (Deinhardt et al. 1967). The agent was isolated and termed as GB virus A (GBV-A) and GB virus B (GBV-B) (Simons et al. 1995b). Recently, GBV-A and GBV-B have been considered the viruses of

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tamarin (Schlauder et al. 1995). GB virus C (GBV-C) (Simons et al. 1995a) and hepatitis G virus (HGV) (Linnen et al. 1996) are now considered the viruses associated with human, and are regarded as a single virus. HGV is a single-stranded RNA virus that can be categorized as family Flaviviridae and that resembles the hepatitis C virus (HCV) (Linnen et al. 1996). Since HGV was isolated from the serum of a patient with acute hepatitis, it is possible that this virus causes hepatocyte injury (Yoshiba et al. 1995). In addition, the similarity of this virus to HCV implies that it may also have similar infection routes. However, the virulence and infection routes of this virus have yet to be determined.

In order to determine the genoepidemiology of HGV infection we examined the HGV in the serum of those presumably at high risk of infection: intravenous drug users (IDUs), individuals with tattoos, and patients with chronic viral hepatitis. We used a reverse-transcription polymerase chain reaction (RT-PCR) assay with nested primers deduced from the nonstructural region 3 (NS3) of HGV, and, from these, isolated and sequenced HGV clones.

MATERIALS AND METHODS

Patients

All subjects were Japanese and had either tattoos, a history of drug abuse, or chronic viral hepatitis. Drug abuse was confirmed by inquiring of those who underwent a medical health examination about a history of drug abuse. Individuals with history of intravenous drug abuse and/or tattoo (with history of either intravenous drug abuse or tattoo, or both) had no history of blood transfusion. Patients with chronic viral hepatitis had been followed in our out-patient clinic for at least one year. Blood samples were obtained with full informed consent. Subject profiles are shown in Tables 1 and 2.

TABLE 1. *Profiles of those with intravenous drug abuse and/or tattoos*

	IDU	Tattoos	IDU with tattoos	Total
Number	25	37	55	117
Age (years)	42.2 ± 1.7 ^a	42.4 ± 1.8	42.7 ± 1.3	42.5 ± 0.9
Sex (M/F)	25/0	37/0	55/0	117/0
ALT (IU/liter)	75.4 ± 19.4 ^a	36.9 ± 6.3	50.1 ± 5.2	51.3 ± 5.3
(range)	(4-478)	(5-228)	(11-193)	(4-478)
Anti-HCV (+)	20 (80.0%)	27 (73.0%)	48 (87.3%)	95 (81.2%)
HCV RNA (+)	18 (72.0%)	24 (64.9%)	39 (70.9%)	81 (69.2%)
HGV RNA (+)	4 (16.0%)	6 (16.2%)	6 (10.9%)	16 (13.7%)
HBsAg (+)	0 (0.0%)	3 (8.1%)	2 (3.6%)	5 (4.3%)
Anti-HBs and anti-HBc (+)	16 (64.0%)	14 (37.8%)	31 (56.4%)	61 (52.1%)

^a Mean ± S.E. IDU, intravenous drug users; ALT, alanine aminotransferase

TABLE 2. *HGV RNA positive rate in chronic hepatitis patients*

	CH-B	CH-C	CH-NBNC ^b	Total
Number	50	87	22	159
Age (years)	41.5 ± 1.9 ^a	46.8 ± 1.3	46.4 ± 3.7	45.1 ± 1.1
Sex (M/F)	35/15	50/37	8/14	93/66
ALT (IU/liter)	55.7 ± 6.6 ^a	121.3 ± 9.5	137.9 ± 29.4	104.3 ± 7.4
(range)	(15–243)	(19–406)	(25–556)	(15–556)
HGV RNA (+)	0 (0.0%)	5 (5.7%)	0 (0.0%)	5 (3.1%)

^aMean ± s.e. ^bHBsAg (–), HBcAb < 50% (× 200), HBV DNA (–), HCV RNA (–) and HCV Ab (–)
CH, chronic hepatitis

Their serum samples were analyzed for anti-HCV (second generation), HBsAg, anti-HBs, and anti-HBc with enzyme-linked immunosorbent assay kit (Abbott Laboratories, Abbott Park, IL, USA). Serum samples were also tested for HCV RNA with an RT-PCR assay (Saitoh et al. 1994) and for HBV DNA with a PCR assay (Niitsuma et al. 1995). Diagnosis of chronic non-B non-C viral hepatitis was made in the absence of serum markers for both HBV and HCV, in the absence of excessive alcohol consumption, after ruling out the possibility of alcoholic, drug induced (Sherlock and Dooley 1994), or autoimmune (Johnson and McFarlane 1993) hepatitis, and in the presence of histologic findings compatible with the diagnosis of chronic viral hepatitis (MacSween et al. 1994). Virologically, non-B was defined as the absence of both serum HBsAg and HBV DNA and anti-HBc titer being less than 50%. Non-C was defined as the absence of circulating anti-HCV and HCV-RNA.

Determination of HGV RNA by RT-PCR

Nucleic acids were extracted from 200 µl of serum as described previously (Saitoh et al. 1994; Niitsuma et al. 1995), and were converted to complementary DNA (cDNA) with a random hexamer. Reversely-transcribed cDNA was subject-

TABLE 3. *Primers used in this study*

Primer	Sequence
G1	5'-GACGTTGGTGAGATCCCCTT-3'
G2	5'-CGAAGTTTCCTGTGTACCC-3'
G3	5'-TGGGCATGGCATMCCCCTGG-3'
G4	5'-ACSAGGTCCCCRTCYTTGATG-3'
G5	5'-GCIACIGCIACNCCNCCNGG-3'
G6	5'-ATGGTIAIIGTNGGRTCHARR-3'

I, Inosine; S, C or G; R, A or G; Y, C or T; M, A or C;
N, A,C,G or T; T, A,C or T

ed to the first round of PCR with primers G1 and G2 (Table 3). PCR was performed with *Thermus thermophilus* (*Tth*) DNA polymerase (Toyobo Co., Ltd., Osaka) for 35 cycles (consisting of denaturation for 1 minute at 93°C, annealing for 1 minute at 55°C, and extension for 1 minute at 74°C) followed by an extension cycle at 74°C for 8 minutes. The second round of PCR was carried out for 30 cycles consisting of the same protocol as those in the first round of PCR. The primers for the nested PCR were G3 and G4 (Table 3). G1 and G2 were the primers constructed by Simons et al. (1995a), and G3 and G4 were originally deduced by us from the nucleotide sequence of an NS3 of HGV described in the GenBank and also sequenced by us. The specificity of G3 and G4 had been confirmed previously (Niitsuma et al. 1996).

Nucleotide sequences of HGV isolates

The PCR products with the primers G1 and G2, and G3 and G4 had 168 base pairs (bp), which was too short for determination of phylogenetic distance between clones of HGV. Thus the PCR products generated by the first round of PCR with G5 and G6 (Table 3) (Simons et al. 1995a) were then amplified by G1 and G2, generating final 240 bp PCR products. Direct sequences of 240 bp PCR products were performed by fluorescence autosequencer (model 373A; Applied Biosystems Inc., Foster, CA, USA) and by Taq Dye Deoxy Terminator Sequencing kit (Applied Biosystems Inc.) as described in the protocols of the autosequencer and sequencing kits. The sequencing primers were G1 (sense) and G2 (antisense).

Phylogenetic analysis of isolated HGV clones

For phylogenetic determination of each HGV clone, the UPGMA method (Nei 1975) was used with the GENETYX-MAC 7.3 (Software Development Co., Ltd., Tokyo). We compared the isolated clones with the 10 reported HGV clones. The GenBank accession numbers of HGV or HCV sequences used in analysis were as follows: U44402, U45966, U25538, U25539, U25540, U25541, U25542, U25543, U25544, U25545, D87255, D87262, D90600, D90601, U67782 and U75356. We also compared these clones with several representative HCV clones: M62321 (genotype 1a), D13558 (genotype 1b), D14853 (genotype 1c), D00944 (genotype 2a), D10988 (genotype 2b), D17763 (genotype 3a) and D49374 (genotype 3b).

Statistical analysis

Results were expressed as mean $\pm$ s.e. Statistical differences of variables between groups were determined by unpaired *t*-tests. *p*-Values below 0.05 were considered statistically significant.

RESULTS

Prevalence of HGV, HCV and HBV infection

In those with history of intravenous drug abuse and/or tattoo ($n = 117$), HGV

TABLE 4. *Serum ALT levels of those with intravenous drug abuse and/or tattoos in relation to serum viral markers*

	Number	Age (years)	Sex (M/F)	ALT (IU/liter) (range)
None	31	39.5 ± 1.8 ^a	31/0	25.9 ± 3.1 ^a (4-87)
HBV alone	2	49.0 ± 2.0	2/0	39.0 ± 24.0 (15-63)
HCV alone	66	44.6 ± 1.1	66/0	63.2 ± 8.6 (14-478)
HGV alone	3	29.3 ± 1.8	3/0	33.3 ± 2.7 (28-36)
HBV and HCV	2	36.0 ± 3.0	2/0	69.5 ± 41.5 (28-111)
HCV and HGV	12	40.9 ± 2.2	12/0	57.3 ± 10.6 (7-120)
HBV, HCV and HGV	1	57.0	1/0	26.0
Total	117	42.5 ± 0.9	117/0	51.3 ± 5.3 (4-478)

^aMean ± s.e.

RNA was detected in 13.7% subjects, HCV RNA in 69.2%, and HBsAg in 4.3% (Table 1). Twelve of 16 individuals with HGV RNA were coinfectd with HCV, and 1 was coinfectd with both HBV and HCV (Table 4). The prevalence of these three viruses (HBV, HCV and HGV) in men with history of intravenous drug abuse, men with tattoo, and men with history of intravenous drug abuse and tattoo was not significantly different (Table 1).

HGV RNA was detected in 5 (3.1%) of 159 patients with chronic viral hepatitis. HGV RNA was detected only in patients with chronic hepatitis CH-C (Table 2), but HGV RNA was not detected in patient with CH-B nor with CH non-B non-C.

The prevalence of HGV in those with history of intravenous drug abuse and/or tattoo was significantly higher than patients with CH-B, those with CH-C and those with CH non-B non-C. The prevalence of HGV in those with CH-C was significantly higher than patients with CH-B and those with CH non-B non-C.

Characteristics of HGV infection

Serum alanine aminotransferase (ALT) level was evaluated in those with history of intravenous drug abuse and/or tattoo. Three individuals who were infected with HGV alone showed normal serum ALT levels (28, 36 and 36 IU/liter). Four (25%) of 12 individuals coinfectd with HGV and HCV, as well as 34 (51.5%) of 66 infected with HCV alone, showed normal serum ALT levels. The mean ALT values of the two groups were 57.3 ± 10.6 and 63.2 ± 8.6 IU/liter and they were not significantly different (Table 4).

In the patients with CH-C, serum ALT levels of those coinfectd with HGV and HCV, and of those with HCV alone, were 87.2 ± 22.4 and 123.4 ± 10.0 IU/liter, and they were not significantly different (Table 5).

TABLE 5. *Serum ALT levels of chronic hepatitis C patients in relation to serum viral markers*

	Number	Age (years)	Sex (M/F)	ALT (IU/liter) (range)
HCV alone	82	46.5 ± 1.4 ^a	47/35	123.4 ± 10.0 ^a (19–397)
HCV and HGV	5	51.2 ± 5.6	3/2	87.2 ± 22.4 (28–155)
Total	87	46.8 ± 1.3	50/37	121.3 ± 9.5 (19–397)

^aMean ± S.E.*Phylogenetic analysis of isolated HGV*

The nucleotide sequences of HGV spanning 199 bp in nonstructural region 3 revealed that the clones isolated from 11 individuals were closely allied to the HGV sequences in GenBank (Figs. 1 and 2), and distinct from HCV (Fig. 2). The numerical value in Fig. 2 showed the phylogenetic index. A phylogenetic tree of the clones isolated in this study and those reported previously showed several distinct 'branches' of the HGV genome (Fig. 2). When the clones were subdivided at point 0.08 of the phylogenetic index, three genotypes were identified (genotypes 1 to 3 as shown in Fig. 2). Genotype 1 was found in Africa, genotype 2 in the USA and several regions worldwide and genotype 3 in Asia including Japan. Further, variation in the phylogenetic index among HGV clones was 0.0951 while that among HCV clones was 0.1744 (Fig. 2).

DISCUSSION

Previous studies have indicated that HGV is transmitted through blood; antibody to this virus was found in 7% of multi-transfused patients and 1.8% of drug abusers (Zuckerman 1995). Whether these patients are coinfecting with HCV or HBV is not known. The direct detection of HGV by PCR has been recently developed, and has revealed HGV in 0.9% of blood donors in Japan and in 3.1–10.1% of Japanese patients under hemodialysis (the patients at high risk for blood-born transmission of the virus) (Masuko et al. 1996; Nakatsuji et al. 1996). In our study, 13.7% of those with history of intravenous drug abuse and/or tattoos had HGV, as did 5.7% of CH-C patient, though the virus was found in none of the non-B non-C CH patients. These results suggest that HGV may not be a major causal factor in cases of non-B non-C CH in Japan. In Italy the HGV genomes were detected in 35% of the acute and 39% of the chronic hepatitis patients of unknown etiology (Fiordalisi et al. 1996). The cause of this difference is unknown.

Fig. 1. Nucleotide sequence of a 199 bp portion of HGV obtained by us, and by the HGV in GenBank. Only deviations from the original sequence identified by Simons et al. (1995a) (HG25538) are shown.

U25538	TTATGGGCATGGTATCCCCCTCGAGCGTATGAGGACTGGTCGCCACCTTGATTCTGCCATTCCAAG
U25539	A.....G.....C.....C.....G.....C.....G.....A.....
U25540	C.....A.....G.....A.....G.....C.....C.....CA.....G.....C.....A.....
U25541	C.....A.....T.....G.....A.....G.....C.....C.....AA.....G.....C.....G.....A.....
U25542	C.....T.....T.....G.....G.....CA.....C.....AA.....A.....T.....G.....C.....C.....G.....
U25543	C.....A.....A.....A.....G.....C.....A.....C.....AG.....G.....C.....G.....T.....A.....
U25544	A.....A.....A.....G.....C.....A.....C.....AA.....G.....C.....G.....T.....T.....
U25545	C.....C.....A.....A.....G.....C.....C.....A.....G.....C.....T.....C.....T.....
U44402	C.....A.....T.....G.....G.....C.....C.....A.....G.....T.....C.....G.....A.....
U45966	C.....C.....A.....A.....G.....C.....A.....C.....AG.....T.....C.....G.....T.....A.....
D87255	C.....A.....T.....G.....G.....C.....C.....A.....G.....T.....C.....G.....A.....
D87262	C.....A.....T.....G.....G.....C.....C.....A.....G.....T.....C.....G.....A.....
D90600	C.....C.....A.....T.....G.....G.....C.....C.....A.....G.....T.....C.....G.....A.....
D90601	C.....A.....T.....G.....G.....C.....C.....A.....G.....T.....C.....G.....A.....
U67782	C.....A.....T.....G.....G.....C.....C.....A.....G.....T.....C.....G.....A.....
U75356	C.....A.....T.....G.....G.....C.....C.....A.....G.....T.....C.....G.....A.....
Pt.1	C.....C.....A.....T.....G.....G.....C.....C.....A.....G.....T.....C.....G.....A.....
Pt.2	C.....A.....T.....G.....G.....C.....C.....A.....G.....T.....C.....G.....A.....
Pt.3	C.....A.....T.....G.....G.....C.....C.....A.....G.....T.....C.....G.....A.....
Pt.4	C.....A.....T.....G.....G.....C.....C.....A.....G.....T.....C.....G.....A.....
Pt.5	A.....C.....A.....T.....G.....G.....C.....C.....A.....G.....T.....C.....G.....A.....
Pt.6	C.....A.....T.....G.....G.....C.....C.....A.....G.....T.....C.....G.....A.....
Pt.7	C.....C.....A.....T.....G.....G.....C.....C.....A.....G.....T.....C.....G.....A.....
Pt.8	C.....A.....T.....G.....G.....C.....C.....A.....G.....T.....C.....G.....A.....
Pt.9	C.....A.....T.....G.....G.....C.....C.....A.....G.....T.....C.....G.....A.....
Pt.10	C.....A.....T.....G.....G.....C.....C.....A.....G.....T.....C.....G.....A.....
Pt.11	C.....A.....T.....G.....G.....C.....C.....A.....G.....T.....C.....G.....A.....
U25538	GCGGAGTGCAGAGATTGGCCGCGCCAGTTCTCCGCGCGGGGGTTAATGCCATCGCCTATTATAGGG
U25539	T.....G.....T.....C.....
U25540	C.....GC.....T.....T.....A.....C.....A.....T.....C.....
U25541	T.....GC.....T.....T.....A.....T.....A.....TG.....T.....
U25542	C.....GC.....C.....T.....T.....T.....T.....A.....A.....C.....T.....T.....C.....
U25543	C.....GC.....T.....T.....T.....T.....A.....C.....C.....T.....C.....
U25544	C.....GC.....T.....T.....T.....T.....A.....G.....T.....C.....
U25545	T.....C.....CC.....T.....T.....T.....T.....A.....C.....T.....C.....
U44402	A.....T.....C.....GC.....A.....T.....T.....T.....TA.....A.....C.....A.....C.....
U45966	T.....C.....TC.....G.....G.....A.....T.....TA.....A.....G.....C.....T.....C.....
D87255	T.....C.....TC.....G.....G.....A.....T.....TA.....A.....G.....C.....T.....C.....
D87262	T.....C.....CC.....T.....T.....T.....T.....A.....C.....C.....T.....C.....
D90600	A.....C.....CC.....T.....T.....T.....T.....A.....C.....C.....T.....C.....
D90601	C.....TC.....A.....A.....T.....A.....T.....C.....C.....T.....C.....
U67782	C.....C.....G.....A.....T.....T.....C.....C.....T.....C.....
U75356	C.....GC.....T.....G.....A.....T.....TA.....A.....A.....T.....C.....
Pt.1	C.....TC.....G.....A.....T.....T.....C.....A.....G.....C.....A.....C.....
Pt.2	C.....C.....G.....A.....T.....T.....C.....A.....G.....C.....A.....C.....
Pt.3	C.....C.....G.....A.....T.....T.....C.....A.....G.....C.....A.....C.....
Pt.4	C.....C.....G.....A.....T.....T.....C.....A.....G.....C.....A.....C.....
Pt.5	T.....C.....CC.....T.....T.....T.....T.....A.....A.....T.....T.....C.....
Pt.6	AC.....C.....T.....T.....T.....T.....A.....C.....A.....T.....C.....
Pt.7	AC.....C.....T.....T.....T.....T.....A.....C.....A.....T.....C.....
Pt.8	C.....C.....T.....T.....T.....T.....A.....C.....A.....T.....C.....
Pt.9	C.....C.....T.....T.....T.....T.....A.....C.....A.....T.....C.....
Pt.10	T.....T.....C.....GC.....A.....A.....T.....T.....TA.....G.....A.....A.....C.....
Pt.11	T.....T.....C.....GC.....A.....A.....T.....T.....TA.....G.....A.....A.....C.....
U25538	GTAAGGACAGTTCCATCATCAAAGACGGAGACCTGGTGGTTTGTGCGACAGACGCGCTCTCTACC
U25539	G.....
U25540	G.....A.....T.....C.....T.....A.....T.....C.....T.....
U25541	G.....A.....T.....C.....C.....C.....C.....T.....T.....A.....G.....
U25542	A.....A.....G.....T.....T.....A.....G.....C.....T.....T.....A.....C.....
U25543	C.....A.....G.....G.....A.....G.....C.....C.....C.....A.....C.....G.....
U25544	C.....C.....G.....C.....T.....G.....C.....C.....T.....T.....G.....
U25545	C.....A.....G.....T.....C.....G.....C.....T.....GC.....A.....C.....
U44402	A.....A.....T.....G.....T.....G.....C.....T.....T.....T.....C.....T.....
U45966	A.....A.....T.....G.....T.....G.....C.....T.....C.....A.....C.....T.....
D87255	C.....A.....G.....T.....A.....G.....C.....T.....A.....C.....T.....
D87262	G.....T.....G.....C.....G.....C.....A.....A.....C.....G.....
D90600	A.....T.....G.....T.....G.....C.....G.....C.....A.....A.....C.....
D90601	A.....A.....T.....G.....C.....G.....T.....C.....G.....
U67782	A.....T.....G.....C.....G.....CA.....C.....C.....T.....C.....G.....
U75356	C.....T.....T.....C.....C.....T.....C.....C.....G.....
Pt.1	C.....T.....G.....C.....C.....T.....C.....T.....C.....G.....
Pt.2	G.....C.....A.....T.....G.....C.....G.....C.....T.....A.....C.....G.....
Pt.3	C.....T.....T.....T.....G.....A.....C.....A.....C.....G.....
Pt.4	A.....A.....C.....A.....G.....T.....G.....C.....G.....C.....A.....G.....
Pt.5	G.....C.....A.....T.....G.....C.....G.....C.....A.....G.....
Pt.6	G.....A.....C.....A.....T.....G.....C.....G.....C.....A.....C.....G.....
Pt.7	A.....A.....C.....A.....T.....G.....T.....G.....T.....C.....G.....C.....T.....A.....C.....G.....
Pt.8	A.....A.....C.....A.....G.....T.....G.....T.....C.....G.....C.....T.....A.....C.....G.....
Pt.9	G.....A.....T.....A.....G.....T.....G.....C.....G.....T.....A.....C.....G.....
Pt.10	G.....A.....A.....G.....T.....G.....C.....G.....T.....A.....C.....G.....
Pt.11	G.....A.....A.....T.....G.....C.....G.....C.....G.....A.....G.....

Fig. 1.

Fig. 2.

Currently, it is not known that infection with HGV causes hepatitis. No experimental evidence for hepatitis due to HGV has been revealed. Patients with fulminant hepatitis due to non-A non-B non-C have been shown to have HGV in their serum (Yoshiba et al. 1995), raising the possibility that HGV may cause hepatitis in the acute phase. However the prevalence of HBV in acute post-transfusion hepatitis was found low (Alter et al. 1997). In our study, individuals who were infected with HGV alone or with HGV and HCV did not show biochemical evidence for HGV-related hepatitis in the chronic phase. Our results were in agreement with the results reported by Tanaka et al. (1996). Taiwanese group reported that the average peak serum ALT activity of 25 patients infected by HGV alone was 31 IU/liter (range, 12 to 123), with persistently normal levels in 20 patients (Wang et al. 1996). A recent study demonstrated the possibility of HGV replication outside the liver (Kudo et al. 1997). Thus, it is speculated that HGV may not cause hepatitis. However, we have learned from HCV infection that even those whose serum ALT levels are within normal range can have histological evidence of chronic hepatitis (Healey et al. 1995; Shindo et al. 1995). Thus, we cannot exclude the possibility that HGV may cause mild hepatitis.

The fragment of HGV genome sequenced in this study was the region encoding helicase (Simons et al. 1995a). This region was reportedly similar in HCV and HGV (Simons et al. 1995a). However, isolated HGV clones showed distinct branches of their phylogenetic trees. Phylogenetic analysis is used to classify the genotypes of a virus (Simmonds et al. 1993). In our study, when the index was set at 0.08 for classifying genotypes, three genotypes (1 to 3) of HGV could be identified (Fig. 2). Genotype 1 was localized in Africa, genotype 2 in the USA and several regions worldwide and genotype 3 in Asia including Japan. Recently, two Asian groups reported genotypes of HGV. By the nucleotide sequences of the 5' noncoding region, the HGV strains from Japanese patients clustered in a group distantly separated from the strains of Africa and North America (Fukushi et al. 1996). Further, by the nucleotide sequences of the NS3 region, Taiwanese clones were reported closely related to the East African clone HG25542 (Kao et al. 1996). Although the length of their sequence (118 bp) was shorter than us (199 bp) and thus correct phylogenetic analysis may be difficult, their results are in agreement with ours. Japanese and Taiwanese clones appear closely related each other, and genotype 2 may be called as Asian type. Recently, Muerhoff et al. (1996) reported HGV genotypes according to the nucleotide sequences of the 5' noncoding region. The genotypes they reported seem compatible to the genotypes we reported; genotype 1 is prevalent in Africa, genotype 2

Fig. 2. Phylogenetic tree of HGV isolated by us and in GenBank. Since HCV is close to HGV phylogenetically, HCV clones reported previously are also shown in this phylogenetic tree.

in north America and Europe, and genotype 3 in Asia. Therefore, it is likely that HGV, as well as HCV (Simmonds et al. 1993; Bukh et al. 1994), has genotypes which are prevalent in certain areas of the world.

Further, variation in the phylogenetic index was smaller in HGV than in HCV (0.0951 vs. 0.1744, respectively). The difference may be the result of the longer history of HCV, the lower replicative activity of HGV, or the smaller immune pressure on HGV. The replicative activity of HGV is currently not known. The amount of HGV in chronic HGV carriers appears to be similar to that of HCV in chronic HCV carriers (Linnen et al. 1996), suggesting that the replicative activity of these two viruses may not differ significantly. Immune pressure, whether caused by cellular or humoral immunity, results in a high frequency of mutation in the region where viral transcript is recognized by T lymphocyte or antibody (Kato et al. 1994). Since the transcript from the region sequenced in this study is not an envelope protein of the virus, it is possible that the low variation in the phylogenetic index indicates a low frequency of immune attack by T lymphocytes. This speculation is in agreement with the absence of serum ALT elevation in HGV infection.

A high association of HGV infection with HCV infection may possibly contribute to a decrease in the number of HGV carriers. The implication of the first generation of anti-HCV-ELISA, in 1990, resulted in a drastic decrease in the risk of HCV infection via transfusion; the seroconversion rate dropped to 1.08% per patient and 0.07% per unit (Takano et al. 1993). However, the screening of donated blood for serologic markers of HCV and HBV has not prevented all cases of non-A non-B non-C posttransfusion hepatitis. There may be still agents other than these known hepatitis viruses causing post-transfusion hepatitis.

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